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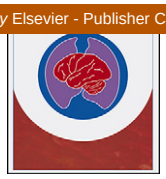

# Effects of oleanolic acid on pulmonary morphofunctional and biochemical variables in experimental acute lung injury

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ABSTRACT

We analysed the effects of oleanolic acid (OA) on lung mechanics and histology and its possible mechanisms of action in experimental acute lung injury (ALI). BALB/c mice were randomly divided into Control (saline, *ip*) and ALI (paraquat, 25 mg/kg, *ip*) groups. At 1 h, both groups were treated with saline (SAL, 50 μl *ip*), OA (10 mg/kg *ip*), or dexamethasone (DEXA, 1 mg/kg *ip*). At 24 h, lung static elastance, viscoelastic pressure, and alveolar collapse reduced more after OA compared to DEXA administration. Tumour necrosis factor-α, macrophage migration inhibitory factor, interleukin-6, interferon-γ, and transforming growth factor-β mRNA expressions in lung tissue diminished similarly after OA or DEXA. Conversely, only OA avoided reactive oxygen species generation and yielded a significant decrease in nitrite concentration. OA and DEXA restored the reduced glutathione/oxidized glutathione ratio and catalase activity while increasing glutathione peroxidase induced by paraquat. In conclusion, OA improved lung morphofunction by modulating the release of inflammatory mediators and oxidative stress.



## 1. Introduction

Lung inflammation is a hallmark of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). The response of cells to lung inflammation may lead to oxidant/antioxidant imbalance, with production of nitric oxide and superoxide and release of cytotoxic and pro-inflammatory compounds, including proteolytic enzymes, reactive oxygen species (ROS), reactive nitrogen species (RNS) and additional inflammatory cytokines, resulting in cellular dysfunction (Chabot et al., 1998; Tasaka et al., 2008) and inhibition of certain lung proteins. This oxidative injury perpetuates inflammation and damages the alveolar-capillary membrane (Lee et al., 2010).

Several pharmacological treatments have been tested to modulate the signalling pathways in order to decrease pulmonary inflammation (Calfee and Matthay, 2007) and restore the oxidant/antioxidant balance (Chavko et al., 2009). So far, however, an effective pharmacological therapy for ALI/ARDS has not been identified. Recently, natural products derived from plant extracts and their synthetic derivatives have been used to treat a wide range of respiratory diseases due to their anti-inflammatory and antioxidative properties. In this line, oleanolic acid (OA), a triterpenoid compound present in a great variety of plants and food products (Liu, 2005), modulates the production and activity of pro-inflammatory cytokines and enzymatic antioxidant defence, as well as protects from oxidant stress by activating Nrf2 (Reisman et al., 2009; Takada et al., 2010; Wang et al., 2010). Chemical synthesis of oleanolic acid has provided many useful derivatives that are more potent and specific than natural parent structures (Honda et al., 1997). Reddy et al. demonstrated that intermittent administration of a synthetic triterpenoid compound, CDDO-imidazole (CDDO-Im) (1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl] imidazole, during exposure to hyperoxia confers protection against the development of ALI in mice (Reddy et al., 2009). However, the effects of oleanolic acid derivatives and triterpene derivatives are not necessarily similar to those of their parent molecules (Honda et al., 1998, 1999). Additionally, even though the biological activity of oleanolic acid is lower than that of its derivatives, it is known to be relatively non-toxic (Liu, 1995, 2005).

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We tested the hypothesis that oleanolic acid may curtail the inflammatory process, improving lung morphology and function in experimental ALI induced by paraquat.

## 2. Materials and methods

This study was approved by the Health Sciences Centre Ethics Committee at the Federal University of Rio de Janeiro. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences, USA.

### 2.1. Animal preparation and experimental protocol

One hundred and eight BALB/c male mice (20–25 g) were kept under specific pathogen-free conditions in the Laboratory of Pulmonary Investigation animal care facility. All animals were randomly assigned to two groups. In the control group (C), mice received saline intraperitoneally (50 μL, *ip*), while in the ALI group paraquat (25 mg/kg, *ip*) was administered. Both groups were further treated with saline [ALI-SAL (0.1 mL, *ip*)], oleanolic acid [ALI-OA (10 mg/kg, *ip*)] or dexamethasone [ALI-DEXA (1 mg/kg, *ip*)] (Göcgeldi et al., 2008) 1 h after paraquat or saline injection, in randomized order. For the present ALI model, different doses of OA (5, 10, and 20 mg/kg animal body weight) were titrated in pilot studies, and the 10 mg/kg dose was chosen based on the lowest mortality rate and lung morphofunction impairment. Thirty-six mice ($n = 6$/each) were used to evaluate lung mechanics and histology, as well as molecular biology. Forty-two animals ($n = 7$/each) were submitted to the same protocol described above to obtain aliquots of bronchoalveolar lavage fluid (BALF). The remaining 30 animals ($n = 5$/each) were used to evaluate the activity of antioxidant enzymes, GSH/GSSG ratio and RNS.

### 2.2. Lung mechanics

24 h after administration of paraquat or saline, the animals were sedated [diazepam (1 mg/kg, *ip*), anaesthetised [thiopental sodium (20 mg/kg, *ip*)], tracheotomised, paralysed (vecuronium bromide, 0.005 mg/kg, *iv*), and ventilated with a constant flow ventilator (Samay VR15; Universidad de la Republica, Montevideo, Uruguay) with the following parameters: respiratory frequency of 100 breaths $min^{-1}$, tidal volume ($V_T$) of 0.2 mL, and fraction of inspired oxygen of 0.21. During spontaneous breathing, the level of anaesthesia was assessed by evaluating the size and position of the pupil, its response to light, the position of the nictitating membrane, and the tone of jaw muscles. After muscle relaxation, adequate depth of anaesthesia was assessed by evaluating pupil size and light reactivity (Correa et al., 2001).

A positive end-expiratory pressure (PEEP) of 2 $cmH_2O$ was applied and the anterior chest wall was surgically removed. After a 10-min ventilation period, lung static elastance (Est,L), resistive ($\Delta$P1,L) and viscoelastic ($\Delta$P2,L) pressures were measured by the end-inflation occlusion method (Bates et al., 1985). All data were analysed using the ANADAT data analysis software (RHT-InfoData, Inc., Montreal, Quebec, Canada). The duration of the lung mechanics data collection was 30 min per animal.

### 2.3. Histology

A laparotomy was done immediately after determination of lung mechanics, and heparin (1000 IU) was injected intravenously in the vena cava. The trachea was clamped at end expiration, and the abdominal aorta and vena cava were sectioned, yielding a massive haemorrhage that quickly killed the animals. The right lung was fixed with 10% buffered formaldehyde solution and paraffin embedded. Four-micrometre-thick slices (3/lung) were cut and stained with haematoxylin-eosin. Lung morphometric analysis was performed using an integrating eyepiece with a coherent system consisting of a grid with 100 points and 50 lines (known length) coupled to a conventional light microscope (Olympus BX51, Olympus Latin America-Inc., Brazil). The volume fraction of the lung occupied by collapsed alveoli (alveoli with rough or plicate walls) or normal pulmonary areas, and the amount of polymorpho- and mononuclear cells and pulmonary tissue were determined by the point-counting technique (Weibel, 1990), made across 10 random non-coincident microscopic fields at a magnification of 200× and 1000×, respectively.

### 2.4. Cytokine mRNA expression using ribonuclease protection assay

Four animals in each group were used for determination of cytokine mRNA expression by using ribonuclease protection assay (RPA).

The *in vitro* transcription kit and a customized template set [containing mouse tumour necrosis factor (TNF)-α, interleukin (IL)-6, interferon (IFN)-γ, transforming growth factor (TGF)-β1, the housekeeping gene glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), and L32 (ribosomal RNA)] were used to synthesize a radiolabeled probe set using [α-$^{32}$P]UTP. Each specific hybridized product migrates according to its size, thereby allowing identification of individual bands that were assigned to specific mRNA products. After RNAse treatment and purification, protected probes were run on a sequence gel, exposed to X-ray films, and developed. The quantity of each mRNA species in the original RNA sample was determined on the basis of the signal intensity (by optical densitometry) given by the appropriately sized, protected probe fragment band. Density of each cytokine mRNA was expressed relative to that of the housekeeping gene GAPDH. These values were then related to control group (Leite-Junior et al., 2008).

### 2.5. Evaluation of bronchoalveolar lavage fluid

In 42 additional animals ($n = 7$/each) reactive oxygen species (ROS) were measured in leukocytes recovered in bronchoalveolar lavage fluid with a flow cytometry assay. For this purpose, a polyethylene cannula was inserted into the trachea and a total volume of 1.5 mL of buffered saline (PBS) containing 10 mM EDTA was instilled and aspirated three times. The bronchoalveolar lavage fluid was centrifuged, and the pellet containing leukocytes was resuspended in PBS. ROS were measured using a fluorescent probe dissolved in DMSO and re-suspended in PBS to a final concentration of 20 μM. Flow cytometry was used to measure intracellular fluorescence. To measure ROS generation, $H_2$DCF-DA (2,7-dichlorodihydrofluorescein diacetate from molecular probes) was used. The fluorescence was measured at the fluorescent (FL)1 channel and the results were expressed as the mean of fluorescence intensity (MFI) (Ka et al., 2003).

### 2.6. Lung tissue analyses

#### 2.6.1. Protein content

In the last set of animals, lungs were homogenized (Homogenizer Nova Tecnica mod NT 136, Piracicaba, Brazil) in 1.0 mL potassium phosphate buffer (pH 7.5), centrifuged at 3000 × *g* (centrifuge FANEM mod 243 M, Sao Paulo, Brazil) for 10 min, and supernatants were collected for biochemical analysis. Protein concentration was estimated by Bradford's protocol, using bovine serum albumin as a standard (Bradford, 1976).

*2.6.2. Nitrite assay*

Nitrite ($NO_2^-$), a by-product of nitric oxide metabolism, was measured with the Griess reaction (Valença et al., 2009). Samples of lung homogenates (100 μL) were reacted with 50 μL of 1% sulphanilamide solution for 10 min and mixed with 50 μL of 0.1% naphthyl ethylenediamine solution. Formation of the purple azo compound was measured spectrophotometrically by absorbance at 540 nm. The method was standardized with increasing concentrations of nitrite, which were expressed as μmol/mg protein.

*2.6.3. Reduced glutathione (GSH) and glutathione disulfide (GSSG) assay*

This assay was based on the reaction of GSH or GSSG with 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), which produces the 2-nitro-5-thiobenzoate (TNB) chromophore (Rahman et al., 2006). To determine GSSG, lung homogenate samples were treated with 2-vinylpyridine, which covalently reacted with GSH (but not GSSG). The excess 2-vinylpyridine was neutralized with triethanolamine. The rate of formation of TNB, measured spectrophotometrically by absorbance at 412 nm, is proportional to the concentration of GSH or GSSG in the sample. The concentration of an unknown sample was determined based on linear equation or the regression curve generated by several standards of GSH or GSSG. The final result was presented as GSH (nmol/mg protein), GSSG (nmol/mg protein), and GSH/GSSG ratio.

*2.6.4. Activity of catalase (CAT) and glutathione peroxidase (GPx)*

CAT and GPx activities were determined in lung homogenates. CAT activity was measured by the rate of decrease in hydrogen peroxide concentration at 240 nm (Aebi, 1984). GPx activity was measured by monitoring the oxidation of NADPH at 340 nm in the presence of $H_2O_2$ (Flohé and Günzler, 1984).

*2.7. Statistical analysis*

The normality of the data (Kolmogorov-Smirnov test with Lilliefors' correction) and the homogeneity of variances (Levene median test) were tested. Since no significant differences were observed between the control groups, only one control group was considered. Thus, differences among the groups were assessed by one-way ANOVA followed by Tukey's test. Survival rates were compared by the log-rank test. Correlations between lung mechanical and morphometric parameters were evaluated using Spearman's correlation test. A $p$ value < 0.05 was considered significant. Data are presented as mean + SEM. The SigmaStat 3.1 statistical software package (Jandel Corporation, San Raphael, CA, USA) was used.

## 3. Results

Survival rate was lower in the ALI-SAL group (60%) compared to the Control group (100%) ($p < 0.001$) and increased in ALI-OA and ALI-DEXA (85%) as compared to ALI-SAL ($p < 0.05$).

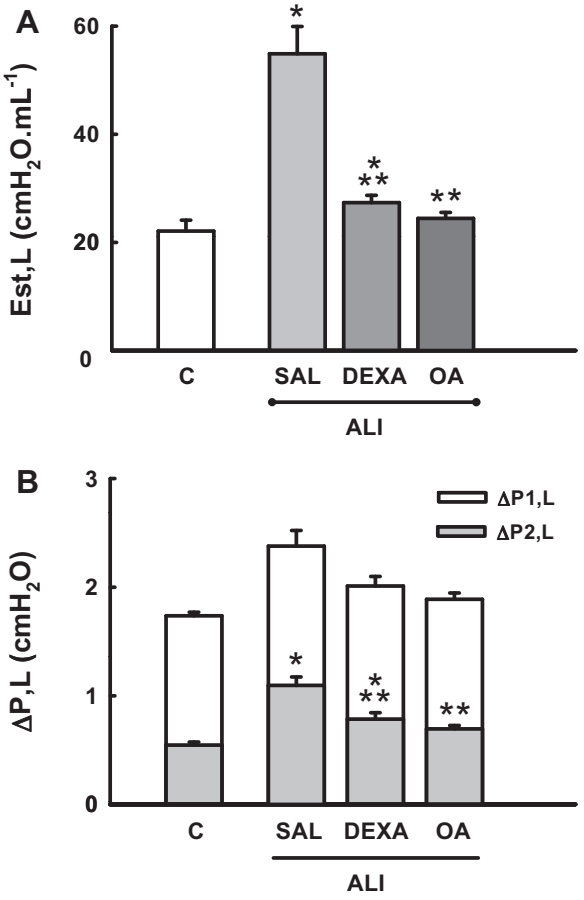


**Fig. 1.** (A) Est,L = lung static elastance, and (B) ΔP1,L = lung resistive pressure and ΔP2,L = lung viscoelastic/inhomogeneous pressure. In control (C) animals, saline was intraperitoneally (50 μL, *ip*) injected. In ALI group, mice received paraquat (25 mg/kg, *ip*). In ALI-SAL, ALI-DEXA, and ALI-OA, animals were treated with saline (0.1 mL, *ip*), dexamethasone (1 mg/kg, *ip*), and oleanolic acid (10 mg/kg, *ip*), respectively, 1 h after the induction of lung injury. Values are means + SEM of 6 animals in each group. *Significantly different from C group ($p < 0.05$). **Significantly different from ALI-SAL group ($p < 0.05$).

Est,L and ΔP2,L were significantly higher in ALI-SAL compared to the Control group (Fig. 1A and B). Mechanical parameters improved after administration of both OA and DEXA, but only the ALI-OA group reached Control levels. No changes occurred in ΔP1,L after induction of ALI or treatment.

The fraction area of alveolar collapse, total cells and neutrophils was higher in ALI-SAL compared to the Control group (Table 1). The fraction area of alveolar collapse was reduced in ALI-OA and ALI-DEXA, but this reduction was more effective in the ALI-OA group.

**Table 1**
Lung morphometry.

| GROUP | Normal (%) | Alveolar collapse (%) | Total cells (%) | Neutrophils (%) | MN (%) |
|---|---|---|---|---|---|
| Control | 93.5 ± 1.0 | 6.5 ± 1.0 | 36.7 ± 1.1 | 0.3 ± 0.1 | 36.1 ± 1.1 |
| ALI | | | | | |
| SAL | 38.8 ± 3.4* | 61.2 ± 4.1* | 43.4 ± 1.0* | 7.5 ± 0.4* | 35.9 ± 1.0 |
| DEXA | 82.9 ± 2.1*,** | 17.1 ± 1.5*,** | 37.7 ± 1.8 | 1.9 ± 0.1*,** | 35.8 ± 1.8 |
| OA | 86.3 ± 1.1*,**,# | 13.7 ± 0.9*,**,# | 37.7 ± 0.8 | 2.1 ± 0.3*,** | 35.5 ± 0.9 |

Values are means (±SEM) of 6 animals in each group. All values were computed in ten random, non-coincident fields per mice. Fraction areas of normal, collapsed alveoli, total cells, neutrophils, and MN (mononuclear cells). In Control animals, saline was intraperitoneally (50 μL, *ip*) injected. In the ALI group, mice received paraquat (25 mg/kg, *ip*). In ALI-SAL, ALI-DEXA, and ALI-OA, animals were treated with saline (0.1 mL, *ip*), dexamethasone (1 mg/kg, *ip*), and oleanolic acid (10 mg/kg, *ip*), respectively, 1 h after the induction of lung injury.

* Significantly different from Control group ($p < 0.05$).
** Significantly different from ALI-SAL group ($p < 0.05$).
\# Significantly different from ALI-DEXA group ($p < 0.05$).

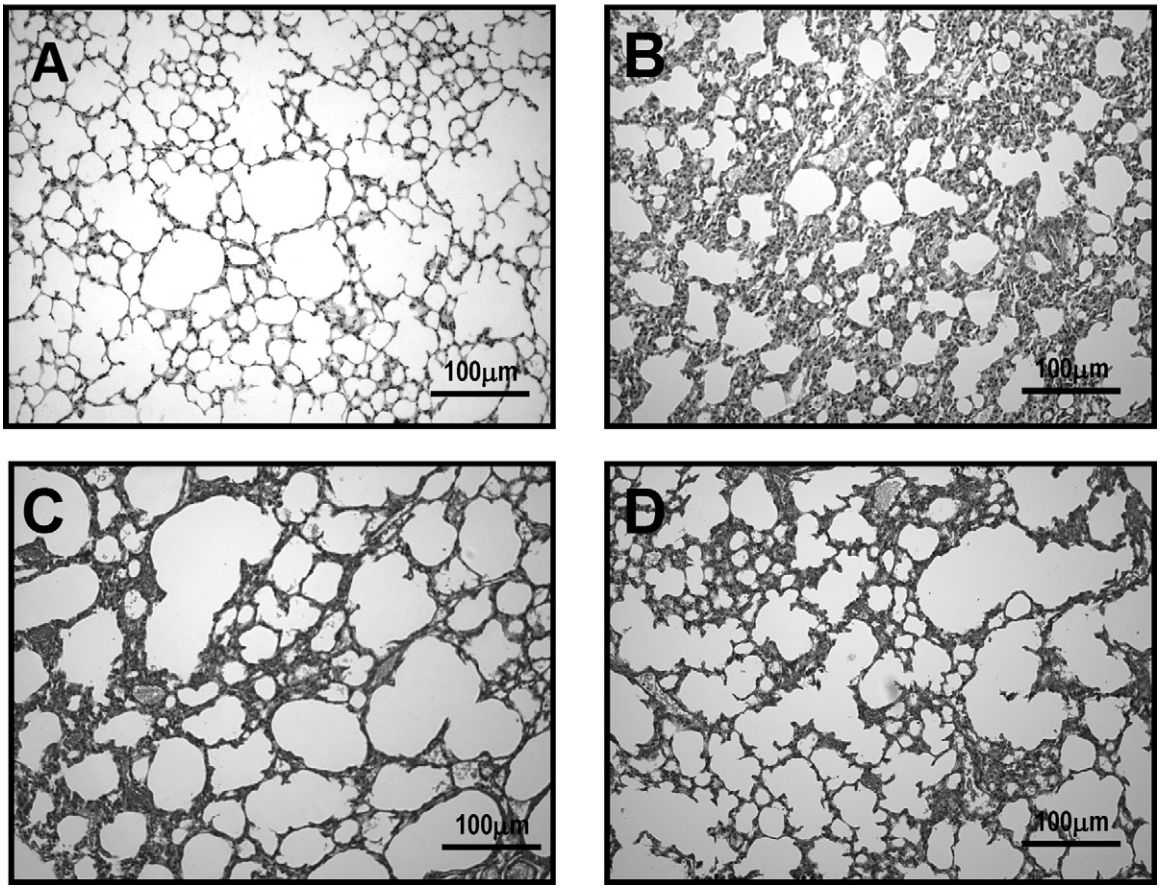


**Fig. 2.** Photomicrographs of lung parenchyma stained with haematoxylin-eosin. (A) control, (B) ALI-SAL, (C) ALI-DEXA, and (D) ALI-OA groups. In control (C) animals, saline was intraperitoneally (50 μL, *ip*) injected. In acute lung injury (ALI) group, mice received paraquat (25 mg/kg, *ip*). In ALI-SAL, ALI-DEXA, and ALI-OA, animals were treated with saline (0.1 mL, *ip*), dexamethasone (1 mg/kg, *ip*), and oleanolic acid (10 mg/kg, *ip*), respectively, 1 h after the induction of lung injury. Scale bar = 100 μm.

A similar decrease was observed in total cell count and neutrophils after OA or DEXA administration (Table 1 and Fig. 2).

Considering all groups, Est,L and ΔP2,L were significantly correlated with total cell count [$r = 0.80$ ($p < 0.001$) and $r = 0.60$ ($p < 0.016$), respectively], and alveolar collapse [$r = 0.88$ ($p < 0.001$) and $r = 0.70$ ($p < 0.003$), respectively].

TNF-α, MIF, IL−6, IFN-γ, TGF-β mRNA expressions were higher in ALI-SAL compared to the Control group. OA and DEXA administration minimized these changes with no significant differences between these therapies (Fig. 3).

In the ALI-SAL group, the MFI of ROS increased significantly compared to the Control group. OA prevented ROS generation more effectively than DEXA (Fig. 4).

Nitrite generation increased in ALI-SAL compared to the Control group. In ALI-OA, but not in ALI-DEXA group, nitrite concentration significantly decreased compared to ALI-SAL (Fig. 5).

As shown in Fig. 6, there was a reduction in GSH/GSSG ratio in ALI-SAL compared to the Control group. GSH/GSSG ratio was restored in the ALI-DEXA and ALI-OA groups (Fig. 6A). The activity of glutathione peroxidase (GPx) was reduced in ALI-SAL compared to the Control group. After DEXA treatment, there was an increase in GPx activity compared to ALI-SAL, but Control levels were not reached. GPx activity was highest after OA administration (Fig. 6B). The activity of catalase (CAT) was elevated in ALI-SAL compared to the Control group. DEXA and OA treatments caused a decrease in CAT activity compared to the ALI-SAL group. Nevertheless, CAT activity returned to Control levels only after OA therapy (Fig. 6C).

## 4. Discussion

In the present study, intraperitoneal administration of oleanolic acid 1 h after paraquat-induced acute lung injury (1) reduced alveolar collapse and neutrophil infiltration, improving lung mechanics, (2) modulated the inflammatory process, diminishing pro-inflammatory cytokines, (3) avoided reactive oxygen species generation and led to a significant decrease in nitrite concentration, (4) modulated the activity of antioxidant enzymes, such as glutathione peroxidase and catalase, and (5) restored GSH/GSSG ratio.

To the best of our knowledge, this is the first study investigating the effects of OA in an experimental model of ALI. We used an ALI model induced by paraquat, which is an herbicide that accumulates predominantly in the lung, causing damage to type I and II pneumocytes, pulmonary oedema and infiltration of inflammatory cells (Rocco et al., 2004). Paraquat promotes oxidant/antioxidant imbalance through generation of the superoxide anion, which can lead to the formation of more toxic ROS and oxidation of the cellular NADPH, causing disruption of important NADPH-requiring biochemical processes and lipid peroxidation (Suntres, 2002). Furthermore, paraquat itself induces intracellular transcription factors such as nuclear factor (NF)-κB and activator protein-1. NF-κB leads to transcriptional activation of many pro-inflammatory genes, including iNOS, several cytokines, and cyclooxygenase-2 (COX-2), all of which exaggerate the inflammatory process. In the present study, we chose specific mediators that are involved in inflammatory and fibrogenic processes in paraquat-

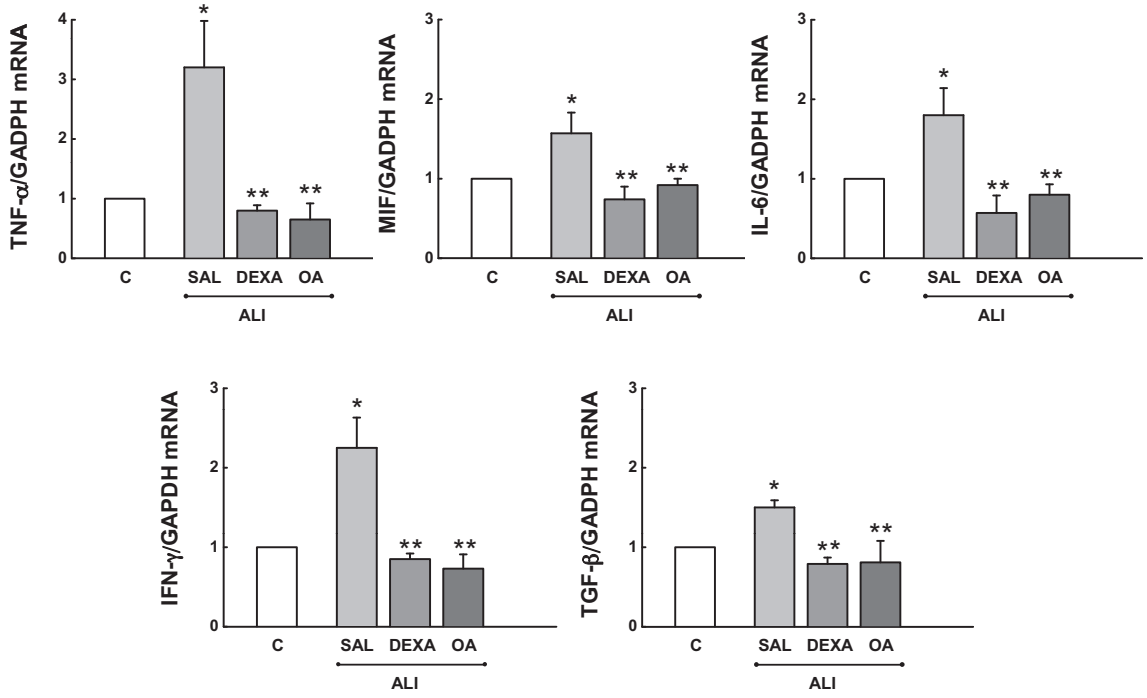


**Fig. 3.** Expression of mRNA for murine cytokines investigated by RNase Protection Assay. The bar graph presents the results (mean + SEM) of four mice per group. These values were then related to control (C) group. In C animals, saline was intraperitoneally (50 μL, *ip*) injected. In the ALI group, mice received paraquat (25 mg/kg, *ip*). In ALI-SAL, ALI-DEXA, and ALI-OA, animals were treated with saline (0.1 mL, *ip*), dexamethasone (1 mg/kg, *ip*), and oleanolic acid (10 mg/kg, *ip*), respectively, 1 h after induction of lung injury. *Significantly different from C group ($p < 0.05$). **Significantly different from ALI-SAL group ($p < 0.05$).

induced acute lung injury, that is, TNF-α, MIF, IL-6, IFN-γ, and TGF-β (Rocco et al., 2004).

Long-term use of a low or moderate dose of OA is relatively non-toxic and safe (Liu, 1995, 2005). The effects of OA were compared with those of an established anti-inflammatory agent, the glucocorticoid dexamethasone at 1 mg/kg (Göcgeldi et al., 2008; Carvalho et al., 2010).

Dexamethasone was used because intraperitoneal absorption of this steroid is more effective than that of other steroids; thus, it is especially adequate for comparison with OA administered intraperitoneally (Engelhardt, 1987). In addition, as a corticosteroid it inhibits NF-κB and activator protein-1, blocks NF-κB-dependent proinflammatory gene expression and the transcription of several cytokines relevant to ALI/ARDS pathology (Jantz and Sahn, 1999), as well as increases intracellular GSH under oxidative stress in alveolar epithelial cells (Rahman et al., 1998). Since the use of corticosteroids has not translated into decreased mortality rates in ALI/ARDS (Diaz et al., 2010), an effort to develop therapeutic agents that act on other inflammatory mechanisms, such as antioxidant activity, is warranted.

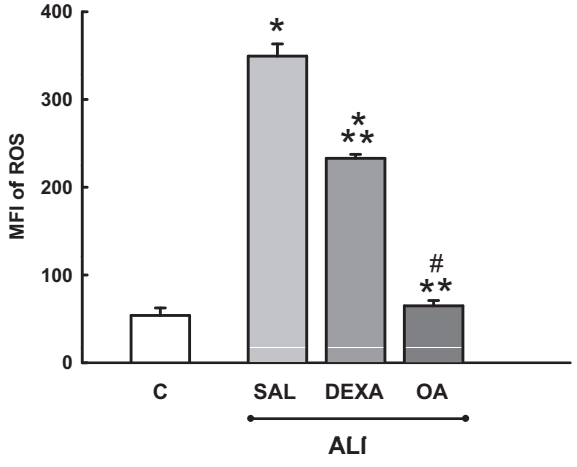


**Fig. 4.** MFI = mean of fluorescence intensity of ROS in bronchoalveolar lavage fluid. In control (C) animals, saline was intraperitoneally (50 μL, *ip*) injected. In the ALI group, mice received paraquat (25 mg/kg, *ip*). In ALI-SAL, ALI-DEXA, and ALI-OA, animals were treated with saline (0.1 mL, *ip*), dexamethasone (1 mg/kg, *ip*), and oleanolic acid (10 mg/kg, *ip*), respectively, 1 h after induction of lung injury. Values are means + SEM of 7 animals in each group. *Significantly different from C group ($p < 0.05$). **Significantly different from ALI-SAL group ($p < 0.05$). #Significantly different from ALI-DEXA group ($p < 0.05$).

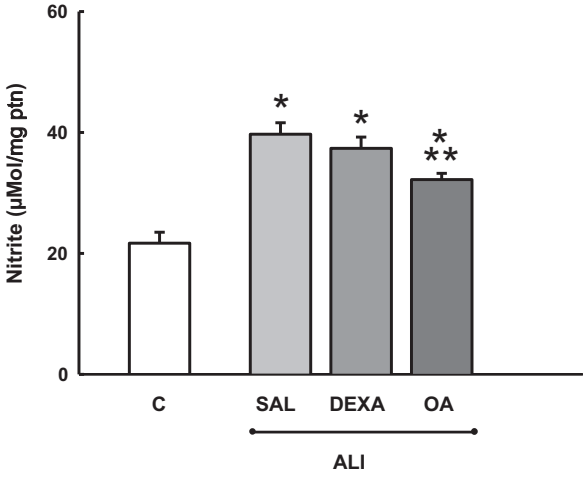


**Fig. 5.** Nitrite concentration in lung tissue. In control (C) animals, saline was intraperitoneally (50 μL, *ip*) injected. In ALI, mice received paraquat (25 mg/kg, *ip*). In ALI-SAL, ALI-DEXA, and ALI-OA, animals were treated with saline (0.1 mL, *ip*), dexamethasone (1 mg/kg, *ip*), and oleanolic acid (10 mg/kg, *ip*), respectively, 1 h after induction of lung injury. Values are means + SEM of 5 animals in each group. *Significantly different from C group ($p < 0.05$). **Significantly different from ALI-SAL group ($p < 0.05$).

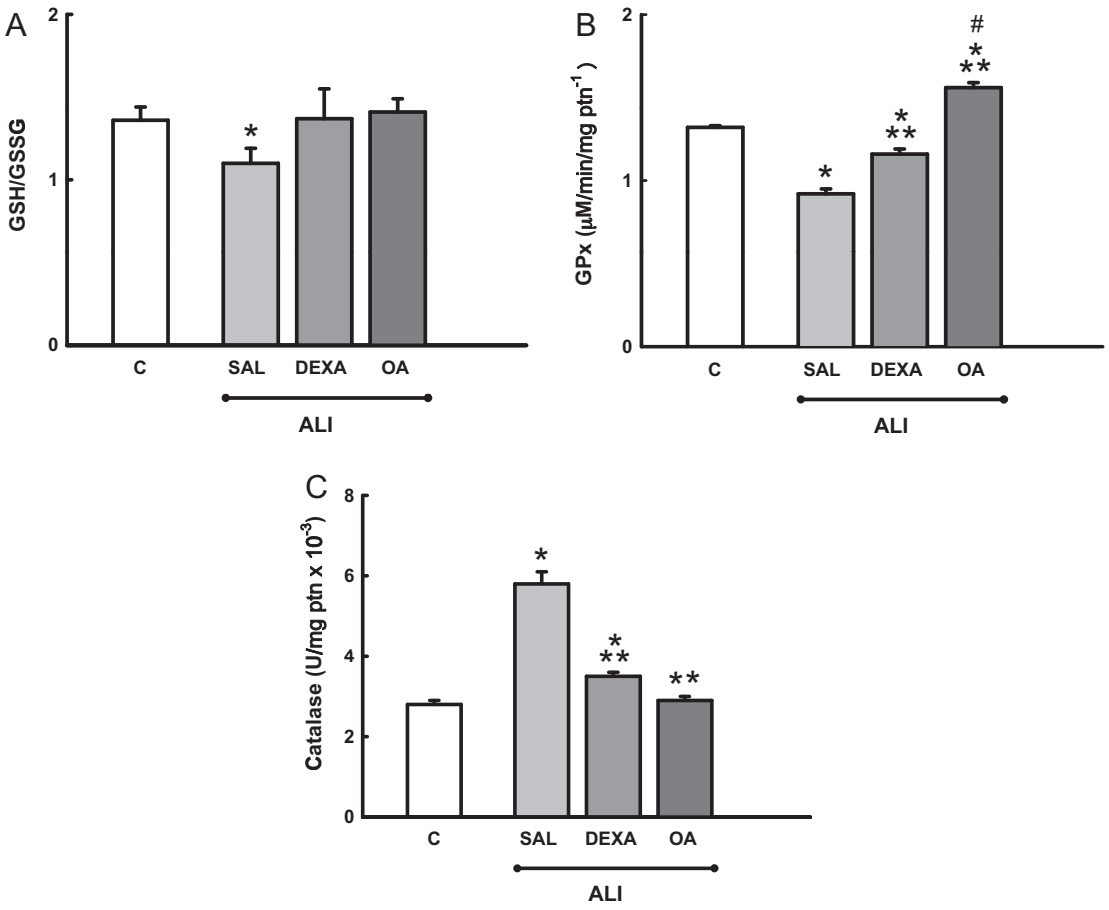


**Fig. 6.** (A) GSH/GSSG ratio. Antioxidant enzyme activities. (B) Glutathione peroxidase. (C) Catalase. In control (C) animals, saline was intraperitoneally (50 µL, *ip*) injected. In ALI group, mice received paraquat (25 mg/kg, *ip*). In ALI-SAL, ALI-DEXA, and ALI-OA, animals were treated with saline (0.1 mL, *ip*), dexamethasone (1 mg/kg, *ip*), and oleanolic acid (10 mg/kg, *ip*), respectively, 1 h after the induction of lung injury. Values are means + SEM of 5 animals in each group. *Significantly different from C group ($p < 0.05$). **Significantly different from ALI-SAL group ($p < 0.05$). #Significantly different from ALI-DEXA group ($p < 0.05$).

In the present study, OA acted on the inflammatory process by reducing generation of pro-inflammatory cytokines (Fig. 3), ROS, and nitrite, as well as by upregulating antioxidant enzymes (Figs. 4–6). Anti-inflammatory effects of OA have been reported (Nataraju et al., 2009; Martín et al., 2010) and associated with inhibition of NF-κB (Takada et al., 2010). This, in turn, has been observed to yield a reduction in inflammatory cytokines and apoptotic cells, as well as nitrite overproduction, with subsequent maintenance of intracellular GSH level (Abdel-Zaher et al., 2007).

Additionally, recent studies have suggested that OA modulates GSH, CAT and GPx activities (Ovesná et al., 2004; Tsai and Yin, 2008; Wang et al., 2010) and exhibits potent scavenging behaviour, with a quenching effect on superoxide anion radicals, preventing redox imbalance and formation of oxidant radicals (Yin and Chan, 2007). It has been proposed that OA may play an antioxidant role through inhibition of the release of high mobility group box-1 protein (HMGB1) (Kawahara et al., 2009) and the activation of Nrf2, a transcriptional factor that induces antioxidant-response elements (Reisman et al., 2009; Wang et al., 2010). A recent study has reported that the targeting of Nrf2 with oleanolic acid derivative may provide an effective therapy to limit the potential adverse effects of hyperoxia (Reddy et al., 2009). However, so far, no study has analysed the impact of oleanolic acid in paraquat induced experimental acute lung injury.

Therefore, the protective effects of OA against ROS in the present paraquat-induced ALI could be associated with a restoration of GSH/GSSG ratio. GSH is a nonprotein thiol that may provide intracellular protection against the oxidative action of paraquat (Tasaka et al., 2008), and also modulate the activity of catalase and GPx (Fig. 6). Furthermore, OA may protect against oxidative stress through iNOS inhibition (Suh et al., 1998), preventing the increase in nitrite, since excessive production of nitric oxide contributes to the pathogenesis of ALI (Lange et al., 2010).

Lung viscoelastic/inhomogeneous pressure and static elastance increased in the ALI-SAL group (Fig. 1A and B) due to alveolar collapse, oedema, and inflammatory cell infiltration (Table 1 and Fig. 2). In the present model, morphofunctional changes were reduced by both DEXA and OA, but these beneficial effects were more intense after OA administration. The mechanical improvement in the OA group was related to a greater reduction in the inflammatory process, which led to less alveolar collapse and oedema. OA and DEXA reduced inflammatory cytokines to a similar degree. However, OA was more effective than DEXA in modulating oxidative stress and regulating the release of nitrite and antioxidant enzymes, such as catalase and glutathione peroxidase. This advantage may be related to the ability of OA to activate nuclear factor E2-related factor 2 (*Nrf2*) and MAP kinases (JNK and ERK) (Wang et al., 2010), while the main role of DEXA is to downregulate NF-κB and AP1 (Meduri et al., 2009).

This study has some limitations that need to be addressed: (1) a specific experimental model of paraquat induced ALI was used. Therefore, the present results may not be extended to other experimental models of ALI, (2) animals were mechanically ventilated in air, and thus we cannot rule out that the increase in inflammatory mediators in ALI-SAL may be related, at least in part, to hypoxia resulting from a greater amount of atelectasis, and/or that different

results could have been obtained with higher $FiO_2$, (3) OA was not compared with a ROS inhibitor but with dexamethasone which has been used in the clinical setting. Thus, we cannot rule out different effects with other types of steroids, different doses and routes of administration, (4) a single intraperitoneal dose of OA was administered, and consequently, we cannot exclude the possibility that multiple doses or continuous infusion could yield better results. The methods to quantify OA in plasma, and the optimal range and route of OA administration in humans are currently being defined (Song et al., 2006; Ji et al., 2009). Even though OA might be safely administered in humans, the optimal oral or intravenous dosage under different clinical conditions remains to be determined, (5) OA was given 1 h after the induction of lung injury, and therefore, the effect of OA at a later phase of ALI is unknown, and (6) OA, but not its derivatives, was used in the current study, thus we cannot exclude that different results could be obtained, and (7) only a limited number of cytokines were investigated, mainly related to inflammatory and fibrogenic processes in paraquat- induced ALI.

In conclusion, intraperitoneal injection of oleanolic acid 1 h after the induction of paraquat-induced acute lung injury modulated the inflammatory and oxidative processes, preventing lung mechanical and histological changes. Thus, oleanolic acid, a drug with anti-inflammatory and anti-oxidative properties, may be a useful adjunct therapy for acute lung injury.

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